

## RESEARCH ARTICLE

### CORRELATION OF MALARIA OUTCOMES AMONG FOUR BASIC ABO PHENOTYPES

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**ABSTRACT: HISTORY:** Clinical reports of Rh blood group and Plasmodium falciparum infection exposes a connection between disease strictness and Rh group. But several studies undertaken have been incapable to link Rh groups to the occurrence of malaria or to the repeat attacks of malaria. **MATERIAL AND METHODS:** This impending study was accompanied for eleven months period from August 2018 to June 2019 on blood samples from patients obtainable with malaria, seen in People's medical college and hospital in Bhopal. In This study comprised all patients diagnosed as malaria positive, seen in People's medical college and hospital. The analysis was based on marginal smear and QBC. Blood group was analysis by forward and reverse method. **RESULT:** The 100 cases included, 61 were positive for plasmodium falciparum and 31 cases were possitive for plasmodium vivax infection. The result of the blood groups showed 21 were blood group , 40 were B, 36 were O, 01 and was AB group. **CONCLUSIONS:** Blood group "O" advantage over the other groups based on artical difference in rosetting ability between RBC different Rh blood group with a diminished rosetting potential in blood group O RBC is suggested as the basis for the differential host susceptibility.

**KEYWORDS:** plasmodium falciparum, plasmodium vivax, RBC, WBC, blood Groups.

**INTRODUCTION:** -Malaria is the parasitic disease of mankind and known since antiquity. The human disease is a protozoan infection of red blood cells transmitted by the bite of a blood feeding female anopheles' mosquito<sup>[1]</sup>. Malaria is a mosquito-borne disease caused by parasites of the genus Plasmodium [P. falciparum, vivax, P. ovale, P. malariae, and P. Knowles] and acquired finished the bites of infected Anopheles mosquitoes' <sup>[2]</sup> malarial parasites are protozoan parasites belonging to the subclass coccidia, genus plasmodium. the genes contains many species which cause malaria in mammals and birds. the asexual cycle of these parasites called schizogony take place in the red blood cell of vertebrates [intermediate hosts] and the sexual cycle, sporgony, occurs in mosquitoes [definitive host].

plasmodium vivax which has the widest distribution, extending through the tropics, subtropics and temperate zones. plasmodium falciparum is also widely distributed, mainly in the tropics. plasmodium malariae occurs sporadically in different parts of the world. plasmodium ovale is mainly confined to central West Africa and some south pacific islands.<sup>[3]</sup>

The are nearly 300 blood group systems so far discovered. the ABO and Rh are the majaor, clinically sinificant and the most important of all the blood group system. all people [with few exception] of ABO system and they are A group, B group AB group and O group.<sup>[4]</sup>

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## LABORATORY DIAGNOSIS

**Stained Blood Films** The blood pictures should be stained as soon as possible as delay may result in stain retention. Romanowsky stains such as Giemsa, Leishman or Picture Leishman's stain has the fixation and staining both occurs at the same time. Therefore, the thick film must be laced before staining by Fleishman stain. In Giemsa and Field stains, on the other hand, the fixative and the tintare separate. Thus, the thin film must be fixed in methanol before staining.

**A Thick blood film.** In a thick blood film, the greatest concentration of blood cells occurs in the center of the film. An area which seems mauve in color [usually near the edges] indicates an area of good color balance between the blue and the red tints, and will show the correct staining characteristics of parasites. in thick films, most of the red blood cells are lysed, and therefore, no longer visible. The blood layer is much thicker than in a thin film, and therefore, one should focus up and down to see the parasites located at different levels.<sup>[5]</sup>

**B. Thin blood film.** Initial screening of a thin film should be carried out with the low-power [10X] objective. Generally, only a few microfilariae occur in a thin blood film preparation and it is possible to miss them if the entire film is not examined. They are commonly found near the edges of the thin film or at the thin end of the film. Once detected, microfilaria should be identified under the oil immersion lens. Near the end of the film, the red cells spread out evenly into a single layer of cells. this area should be examined under the oil immersion lens for malarial parasites, trypanosomes, leishmania etc. the morphology of the red cells and that of the parasites is most clearly seen in these areas making their identification easier, particularly for Plasmodium falciparum infection.<sup>[6][7][8]</sup>

## Approximation of parasitemia by Using Blood Films:

One of the most significant features of reporting malaria infections from lab diagnostic methods is the material gained from approximating the level of parasitemia present in a blood film. A morphological valuation of the parasites is also dangerous to accurate clarification, mainly noting growth stages and the company of hemozoin pigment-containing asexual parasites when reportage *plasmodium Falciparum* infections.<sup>[10]</sup>

**Examination of thin blood films:** examine for at least 101 fields to determine whether the blood film is positive or negative for malaria comperation of RH group.

**FLUORESCENCE ASST;** - Florescence is the event exhibited by certain molecules compounds known as flour chromes. they absorb light energy of 1 wavelength & emit the light energy of another wavelength. If flour chrome receiver the invisible UV light, it will emit the light within the visible spectrum, eg. Green.<sup>[9]</sup>

Fluorescence is used as an isothiocyanate which makes it result in combing the Ag & Ab. When fluoresce in isothiocyanate is combined with antigen and antibody .it is referred to as a conjugate, Fluoresce conjugated with various antigen and antibody are available in market.<sup>[11][12]</sup>

**The centrifugal Quantitative Buffy Coat or QBC:** Buffy coat in the tube examin directly unde a fluorescent Microscope.acridine orangestained malarial parasites appear brilliant green.the qualitative buffy coat is more method than thick blood smear.<sup>[13]</sup> this technique is employed when the number of parasites in blood is low. Buffy coat is a layer of white cells between the red at the bottom and plasma at the top resulting from centrifugation of whole citrated blood.<sup>[14]</sup>

The Buffy coat gives excellent concentration of plasmodium vivax parasitized red cells just below the white cells and platelets. Other

species of malarial parasites do not concentrate so well. The qualitative buffy coat fluorescence method is the more technically demanding and requires specialized equipment to separate the cell layers by centrifugation and a good fluorescence microscope with a high-intensity mercury vapour or quartz halogen lamp to provide the excitation wavelength [15][16]. Although alcidine orange is a very intense fluorescent stain, it is nonspecific and stains nucleic acids from all cell types.

**Polymerase chain reaction:-** Chloroquine is used for treatment of acute malaria. The laboratory procedure called polymerase chain reaction [PCR] is one of the most significant achievements in molecular biology. With this procedure, it is possible to make several copies of one or more genes from very tiny initial amounts of DNA. Large quantities of DNA can be made from very small amounts present in substances such as water, food, blood, hairs, and a host of clinical samples. These are sensitive and specific diagnostic methods for the diagnosis of malaria. It can detect even a low parasitemia. [17][18]

#### Immunochromatographic method:

Chromatography is a process of separation of a mixture of solutes dissolved in a common solvent. Immuno chromatography assay is the test to determine the positive or negative of the sample being analysed. It is commonly named as lateral flow test, is a paper-based platform for the determination. It is a combined form of chromatography and immuno chemistry, the widely used method in form of test strip. [19]

Figure.1

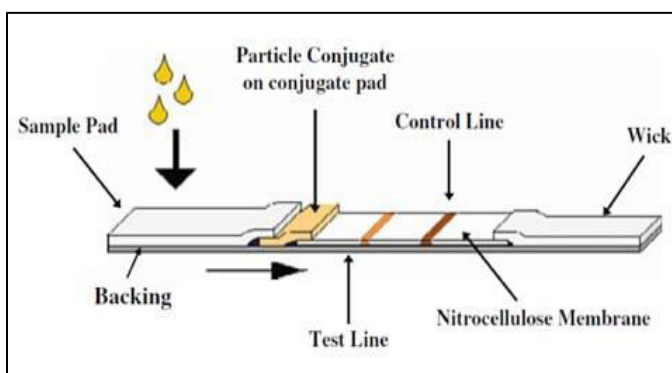


Figure.1 Immunochromatographic method

**Competitive Method-** It is also known as inhibition ELISA or competitive immunoassay; this assay measures the concentration of an antigen by detection of signal interference. The sample antigen competes with a reference antigen for binding to a specific amount of labeled antibody. The reference is pre-coated on a multi well plate. **Figure. 2**

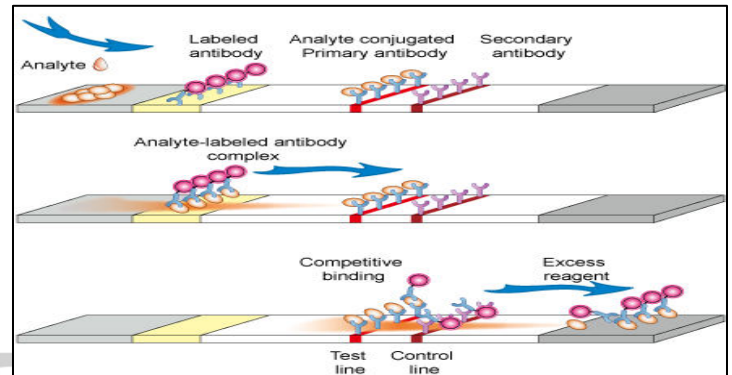


Figure. 2 Competitive Method

**Sandwich Method:-** The sandwich enzyme linked immunosorbent Assay is a sensitive and robust method which measures the antigen concentration in an unknown sample. The antigen of interest is quantified between two layers of antibodies. The capture and detection antibody. **Figure. 3**

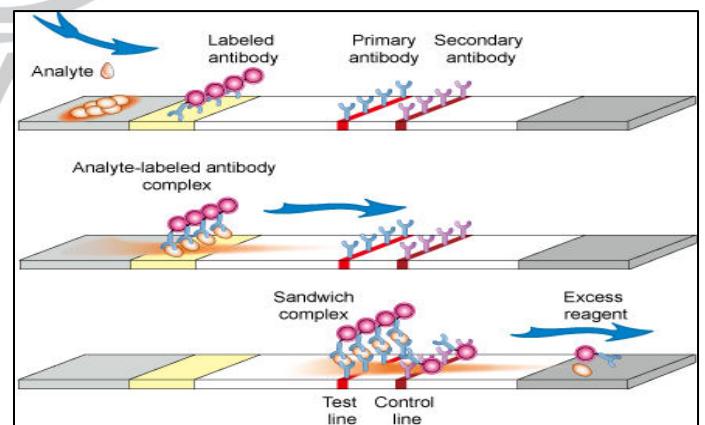


Figure.3 Sandwich Method

**Table. 1 Comparative feature of clinical manifestations of malaria caused by Plasmodium vivax and Plasmodium falciparum**

Character	Plasmodium falciparum	Plasmodium Vivax
Incubation Period	7-27 day, average 12 days	8-31 day, average 14 days
Pre-patent Appearance gametocytes after parasite patency	5 days	8 days
Paresitemia	More then 1.000.000/cc	25.000/cc
Duration of attack	10-12 days	18-20 days
Relapse	Does not occur	Occur
Reerudeseene	Occur	Does not Occur
Drug Resistance	Yes	No

## MATERIAL AND METHODS:

### GENERAL

This prospective study was conducted for eleven months period from August 2018 to June 2019 on blood samples from patients presented with malaria, seen in People's medical college hospital. This study included all patients diagnosed as malaria positive, seen in People's medical college hospital. The diagnosis was based smear and QBC. Blood group was determined by forward and reverse method.

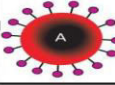
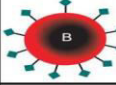
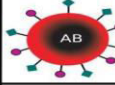




### ABO BLOOD GROUP

- The presence of A, B or O antigens on red cells is determined by the inheritance of the allelic genes A,B and O on chromosome9, which are inherited in pairs as mendelian dominants.
- The O gene produces an inactive transferase, so that H substance persists unchanged in group O.<sup>[20]</sup>

**Table 2 Forward and Reverse grouping**

Forward typing		Group Interpretatio n	Reverse Typing	
Anti A	Anti B		A 1 Cells	B Cells
++++	Negativ e	A	Negativ e	++++
Negativ e	++++	B	++++	Negativ e
++++	++++	AB	Negativ e	Negativ e
Negativ e	Negativ e	O	++++	++++

**Table. 3 Diagrammatic representation of forward and reverse grouping**

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies present			None	
Antigens present	A antigen	B antigen	A and B antigens	None

### Procedure:

- Small drop of blood was placed in the centre of a slide, about 1cm from one end.
- By using a spreader at an angle of 45°C the blood was spread with the width of the spreader with a length of 3 cm.
- Dried the smear and stained with leishman stain.

### Thick Film:

#### Procedure:

- A large drop of blood was taken on the centre of a slide.
- With the help of a spreader the drop was well spread on the slide to about 1.5cmsquare.
- The smear was allowed to dry.
- The thickness showed such that printed matter can just be seen through the film.
- The dried smear was kept in distilled water for 10 minutes for dehaemoglobinization.
- Then the smear was taken out and air dried and stained with Leishman stain. **Figure. 4**



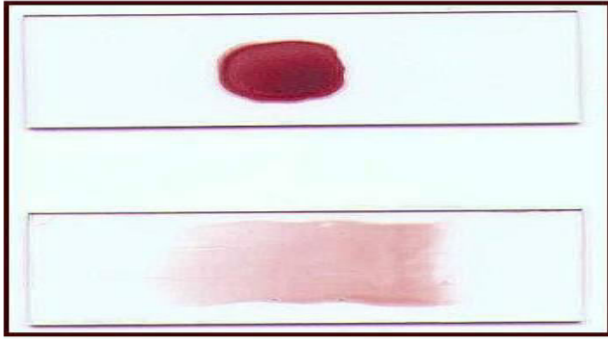


Figure. 4 Preparation of peripheral smear

Stained by Leishman's smears were done after QBC and the species of the malaria parasite was confirmed. After that chart for identification of *plasmodium falciparum* and *plasmodium vivax*. The chart which is used for reference is given as Figure 5

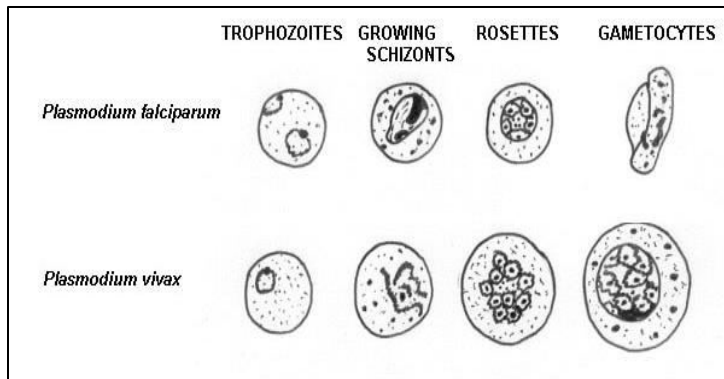


Figure. 5 Chart for identification of Plasmodium falciparum and Plasmodium vivax

## RESULTS:

### General

For eleven months period from August 2018 to June 2019, this prospective study was carried out. Among 1552 smears screened during this period, 100 patients turned out to be positive. 100 samples were evaluated by both QBC method and thick, thin Leishman-stained smears. The species of malarial parasites were *Plasmodium falciparum* [PF][62%] and *Plasmodium vivax* [PV] [38%].

According to blood group VS P. Falciparum is blood group O is 23%, A 22%, AB 3% and blood group B is 52%. **Figure.6**

### BLOOD GROUP VS P. FALCIPARUM

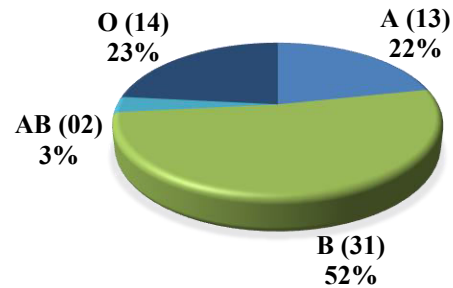


Figure. 6 Blood group and malaria Plasmodium Falciparum

According to blood group malaria parasite plasmodium vivax blood group O is 33%, blood group A 22%, and B blood group is 45%. **Figure.7**

### BLOOD GROUP VS P.VIVAX

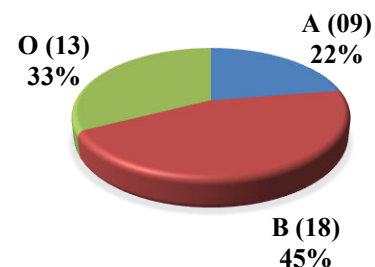
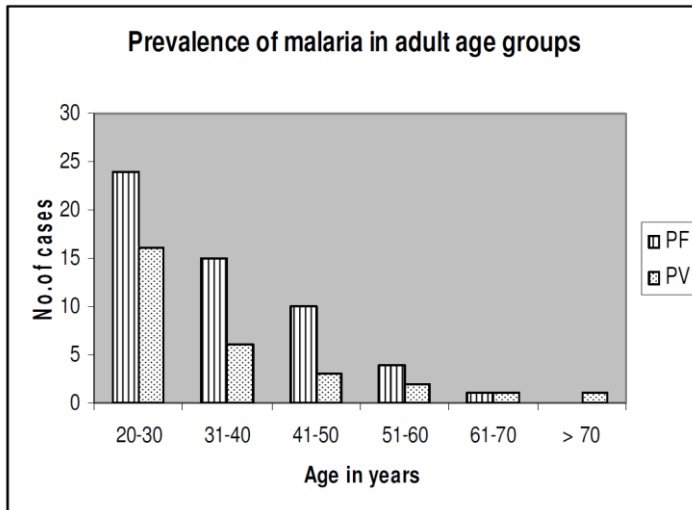


Figure. 7 Blood group and malaria Plasmodium vivax

Regardless of the blood group, the number of patients affected by PF was more than PV. The only patient who had AB positive group had PF and was hospitalized in ICU. Among the patients who had mixed infection 8 had A group, 5 had B group and 2 had O group.

### Age:

Malaria affects all age groups and the age ranged from 1 year to 71 years. Number of adults affected were more [n=84] than children [n=16]. Among the adults the prevalence was more in the younger age group. 68 cases were positive for *Plasmodium falciparum* and 32 cases were positive for *P. Vivax* infection, 14 patients had mixed infection. **Figure. 8**



**Figure. 8** Prevalence of malaria in adult age groups

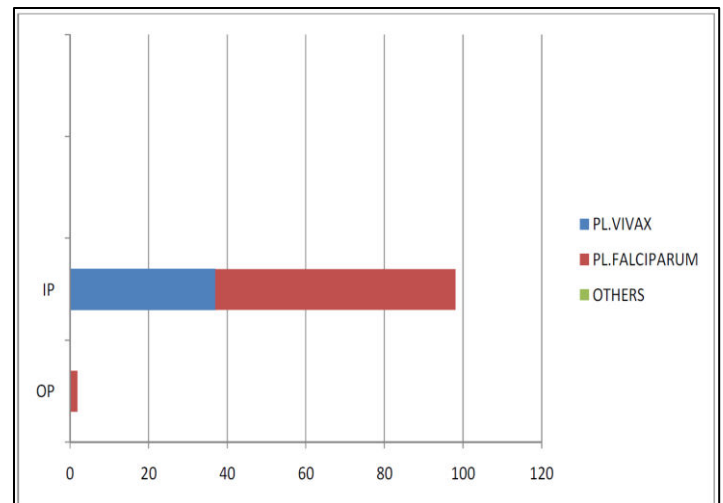
Out of 18 patients in the paediatric age group, 5 of them were treated in ICU, while the rest were treated as in patients. 1 child patient which was one year old, although affected by *P. Vivax*, was in ICU care as the haemoglobin was low [4.4 gm%].

### Gender

Among one hundred patients screened 80 were males and 20 were females. Irrespective of the gender, incidence of PF was more [PF 50/80 males and 13/20 females]. Among males 30 were affected by *Plasmodium vivax*, 07 females were affected by *P. Vivax*.

### OPD and IPD patients

Majority of them were in patients [96%] while only 4 were managed as outpatients, both affected by *Plasmodium falciparum*. Among the 98 hospitalised patients 39 were affected by *Plasmodium vivax* and 59 were affected by *Plasmodium falciparum*. **Figure. 9**



**Figure. 9** OPD and IPD patient ratio

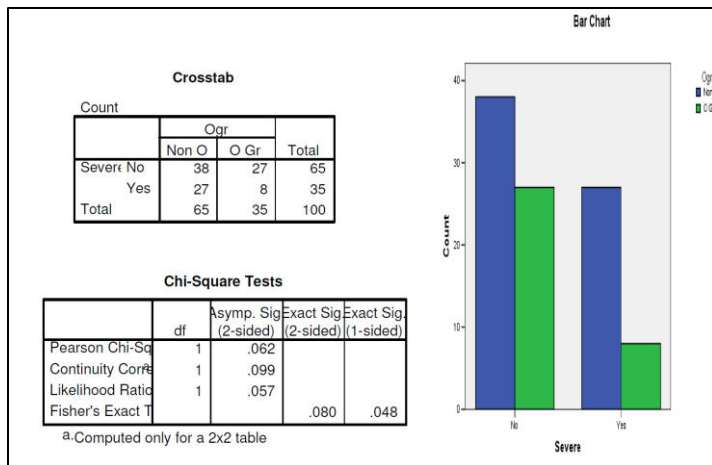
### Clinical Severity:

To compare the clinical course between the different groups, severe infection was defined as when the patient had any two of the following features:

- I. Parasitic load > 10/1000 RBCs.
- II. Severe anaemia with haemoglobin < 6gms%.
- III. Platelet count of < 10,000/mm<sup>3</sup>.
- IV. Hepatomegaly / Splenomegaly.
- V. Clinical signs of severe malaria such as fever >101°F and another organ involvement blood group offers protection compared with non-O blood group.<sup>[21][22]</sup>

• Severe malaria is defined as

- Hyperparasitemia >10% parasitaemia;
- Severe anaemia with Hb < 6.0 g%
- Clinical signs of severe malaria
- Both Hepatomegaly & Splenomegaly,
- Or Thrombocytopenia<sup>[23]</sup>



**Figure .10 Comparison of O and non-O blood group**

After applying Chi square test, it was observed that O blood group offered protection as severity of the disease was less as compared to non-O blood groups. When it was further categorized, the severity was less in A blood group than in B blood groups.<sup>[24][25]</sup> **Figure. 10**

**Table. 4 Summary of the salient laboratory features and blood group phenotype Details ABO**

Details		A	B	O
Number affected	PF	14	27	21
	PV	8	15	14
Adult	PF	12	23	14
	PV	8	13	7
Hb range [in gms%]	PF	5.2-15.2	6.7-13.9	6.5-14.6
	PV	7.6-13.5	8.9-14.1	7.4-14.4
Total WBC Count [per mm <sup>3</sup> ]	PF	2.300-24.000	2.900-28.700	3.500-11.400
	PV	3.200-9.800	3.200-6.400	2.800-10.000
Platelet Count [per mm <sup>3</sup> ]	PF	10.000-1.4 Lakhs	9.000-2.6 Lakhs	13.000-2.8 Lakhs
	PV	35.000-1.3 Lakhs	29.000-1.7 Lakhs	13.000- 1.7 Lakhs

## DISCUSSION:

### General

Malaria has been known since ancient times. The correlation of here dietary indicators with malaria has been the topic of numerous investigations, since Allison showed that the protection provided by sickle cell Hb against infection by *P. Falciparum*<sup>[26]</sup>. Many new data has become apparent from the time when a relationship between Rh blood group& malaria was first optional more than 40 years ago. But the connection of seriousness of malarial infection to the patient's blood group has been of current interest in the search for the answers to the issues affecting clinical course of the disease. The observation by Miller et al. that human RBCs lacking the Duffy blood group antigens are noncompliant to invasion by *Plasmodium vivax* parasites suggests the effectiveness of studying the relationship of blood group with malaria<sup>[27][28]</sup>. Wood noticed that *Anopheles* mosquitoes, which transmit malarial infection, tend to bite persons with blood group O and B in preference to those having group A. In the Indian setup, the literature relating to malaria and the blood groups are sparse and have varied results. Thakur and Verma in their study, they established that Rh blood groups do not show differential vulnerability to malaria<sup>[29]</sup>. In a met analysis of published reports, Singh et al. validated the higher susceptibility to malaria of individuals with blood group A. In contrast Joshi et al reported no correlation between Rh blood groups and malaria in Delhi. The present study tries to associate the blood groups and the clinical presentations in malaria patients and to analyse for differences in the outcome between various blood groups to understand the differential host predisposition in malaria.<sup>[30]</sup>

### Demographics

The study established among the adults; different age group revealed no significant connection with incidence of malaria. All age groups were affected and both genders were affected. However, the progression of the disease in children was severe as compared to

adults. In a cross-sectional study by Eli et al, observed impact of age and other factors that affect clinical consequence of *P. falciparum* malaria in non-immune patients was evaluated<sup>[31]</sup>. Out of 111 patients with *P. falciparum* malaria, 81 [64%] were <37 years old, & only 5% of the patients in this age group developed severe malaria as compared with 18% of the subjects who were 37 years of age [chances ratio, 4.29]; moreover, all deaths happened in the latter group. Male patients did not differ from female patients regarding severity of disease. A similar trend was seen in the present study where the severity did not differ between males and females. RBC of children with severe malaria-associated anemia [SMA] have acquired deficiencies in the complement regulatory proteins complement receptor 1 [CR1, CD35] and decay accelerating factor [DAF, CD55]. Deficiencies in recall CR1 and CD-55 in children with SMA were accompanied by a noticeable decline in immune complex binding capacity and increased cd36 deposition in vivo and ex vivo.<sup>[32][33]</sup> Most importantly, these changes were specific because they were not seen in red cells of children with cerebral malaria or their controls. These data indicate that the declines in red cell CR1 and CD55 seen in children with SMA are of physiologic importance and may predispose RBCs to complement-facilitated damage and in vivo phagocytosis.<sup>[34][35]</sup>

### **CONCLUSIONS:**

Out of 1552 smears screened during a period of 11 months, 100 cases turned to be positive for malaria. Among them, 63 were affected by *P. falciparum* and 37 were affected by *P. Vivax* 98% were hospitalized. On correlation between the blood groups and clinical severity O blood group had a benefit over the other groups. Based on literature difference into setting ability between red cells of different ABO blood groups with a diminished setting potential in blood group O red cells is recommended as the basis for the differential host vulnerability.

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